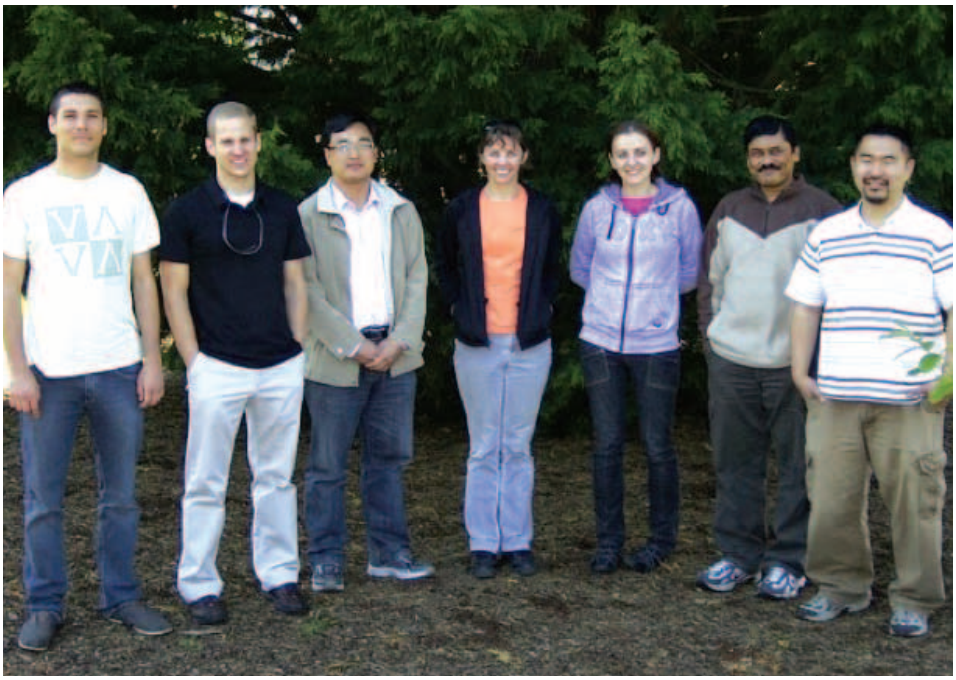
# Behind The Scenes: Changing the "Culture" of Weed Control

*by Ms. Amy Keller*

*The recent issue of the* Journal of Natural Products *included an article from ASP member Dr. Kerry McPhail and her team entitled, "4-Formylaminooxyvinylglycine, an Herbicidal Germination-Arrest Factor from* Pseudomonas *Rhizosphere Bacteria."* The Newsletter *interviewed Dr. McPhail, who took time out of her busy schedule to tell us more about this exciting research on pseudomonad bacteria. Please read the full article in the* Journal of Natural Products, *2010, 73, 1853-1857.*



DR. JONGTAE YANG

The McPhail Team: Mr. Oliver Vining, Mr. Chris Thornburg, Dr. Dahai Zhang, Dr. Kerry McPhail, Ms. Justyna Sikorska, Dr. Murali Thimmaiah, and Dr. Tak Suyama.

**How did you become interested in pseudomonad bacteria?**

As a research assistant professor, I had become aware of agricultural research in Dr. Joyce Loper's laboratory on the Oregon State University (OSU) campus in Corvallis, Oregon, that highlighted the complexity of plant-pseudomonad interactions. Thus, I was hooked when I was approached for help with molecular structure elucidation by Professors Emeriti Donald Armstrong and Dallice Mills, professors of Botany and Plant Pathology at OSU, who presented an intriguing story of a mystery bioactive component in the culture filtrate of certain pseudomonad strains! Unexpectedly, the short structure elucidation project I anticipated turned into a long-term collaboration once the chemical class of the structure was revealed.

**Who in the laboratory carried out the research?**

I performed the structure elucidation of the germination-arrest factor (GAF) by NMR and MS, and the capillary NMR work on the library of *Pseudomonas* isolates. Professor Emeritus Don Armstrong carried out the original GAF purification and also the enzyme assays with D- and L-amino acid oxidase. United Stated Department of Agriculture (USDA) biologist Dr. Mark Azevedo developed the grass seed (*Poa annua*) assay, and cultured and tested the original *Pseudomonas* WH6 strain for isolation and purification of GAF, as well as the library of pseudomonad isolates. Graduate student Chris Thornburg prepared some Pseudomonas WH6 cultures. Graduate student Justyna Sikorska helped to troubleshoot the capillary NMR probe system on occasion. In addition, postdoctoral associate Dr. Murali Thimmaiah has been working on the chemical synthesis of GAF analogs for biosynthetic studies and biological activity testing.

**Could you provide a brief explanation of the work and results in your own words? In what way are the data in your paper new?**

This project to look for potential herbicidal biocontrol agents or natural products was originally initiated in response to the appearance of diuron-resistant weed grasses and also to restrictions on field burning in Oregon. Thus, pseudomonad rhizosphere bacteria were isolated and selected for their ability to arrest germination of annual bluegrass weed (*Poa annua*) seeds. The herbicidal action observed was remarkable because exposed

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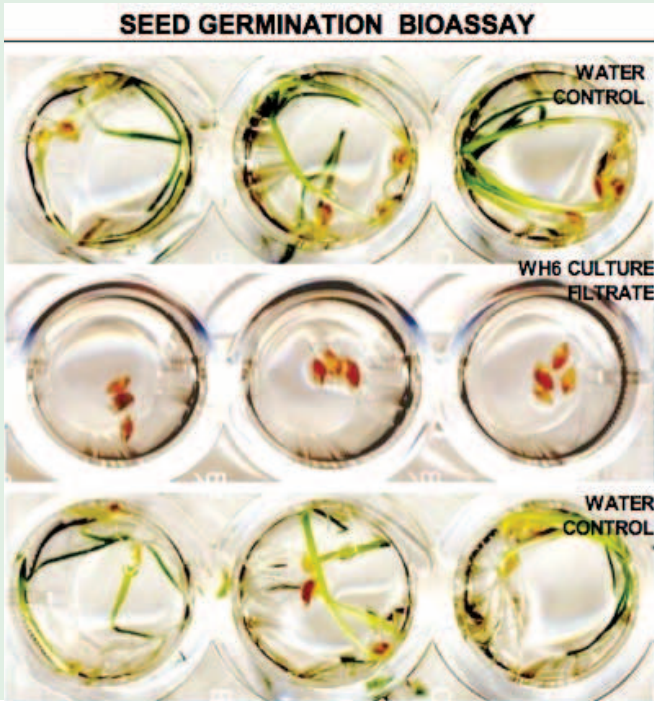
LEFT
**A typical *Pseudomonas* culture bottle.**

RIGHT
***Poa annua* seed germination assay.**




DR. MARK AZEVEDO

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seeds started to germinate before their growth was arrested irreversibly.

In contrast, most herbicides that act on weed seeds are "pre-emergent" in that they prevent the seeds from germinating at all. In addition, the growth of established grass plants and germination of the seeds of broadleaf plant species (dicots) were not affected. The germination-arrest factor (GAF) responsible for this developmentally-specific herbicidal action was purified after an extended effort to isolate it intact from the culture filtrate of *P. fluorescens* WH6. This highly polar, rather labile small molecule was finally assigned as 4-formylaminooxy-vinylglycine on the basis of NMR spectroscopic and mass spectrometric data. D/L-amino acid oxidase assays on culture filtrate extracts were used to establish the L configuration of GAF, which is both tedious to purify continually and not readily accessible via chemical synthesis.

The aminooxyvinyl motif is very unusual and this is the first report of an aminooxyvinylglycine in the English language natural products literature. The oxyvinylglycine motif of GAF suggested that related compounds may exhibit similar herbicidal (and other previously unrecognized) activities. It also suggested that the mechanism of action of GAF is to inhibit pyridoxal phosphate-dependent enzymes, as seen for other vinylglycines. Finally, culture filtrates from the USDA collection of soil bacteria were screened using the grass seed germination assay, analytical TLC, and capillary NMR spectroscopy to show that GAF is secreted by all other herbicidally-active rhizosphere bacteria in our collection. This profiling of isolates was facilitated by the simplicity of the culture filtrates; GAF is fortunately secreted by pseudomonad bacteria grown in minimal culture media, and these could be analyzed with minimal processing.

**What impact does this research have on agriculture or natural products in general?**

Grass seed production is a mainstay of Oregon agriculture. The need for alternatives to field burning as a means to control annual weed grasses that contaminate crop grasses has been emphasized since 1969, the year of "Black Tuesday" when the Oregon State Board of Health received thousands of health-related complaints about smoke from the field burning.

**What is a favorite nonscientific activity of your lab?**

Mine is summer lunch-time sandwiches outside our building near the neighboring field of horses! Otherwise, the students seem to enjoy finding and posting lab cartoons to reflect current events!

**What is your lab's motto?**

Expect the unexpected!

**What is your greatest extravagance in the lab?**

New NMR tubes for 700 MHz! ■